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Allelopathic Aquatic Plants for Aquatic Weed Management

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Abstract. This report presents results of a feasibility study of use of allelopathic aquatic plants for aquatic weed management. In order to establish a list of potential allelopathic plants, we selected 16 aquatic plants native to the southeastern United States and subjected them to two bioassays – one involving lettuce seedlings and one involving the aquatic plant *Lemna minor* as the target species. The lettuce seedling bioassay was selected because it is a widely used, experimentally simple assay to determine allelopathic activity. However, it uses lettuce, a terrestrial plant, as the target species, and thus may be less appropriate for use with aquatic plants. The *L. minor* assay involves an aquatic plant as the target species and so is more appropriate for our goals, but it is experimentally much more complex and time-consuming. The plants selected for study were *Brasenia schreberi, Cabomba caroliniana, Ceratophyllum demersum, Eleocharis acicularis, Eleocharis obtusa, Hydrilla verticillata, Juncus repens, Limnobium spongia, Myriophyllum aquaticum, Myriophyllum spicatum, Najas guadalupensis, Nymphaea odorata, Nymphoides cordata, Potamogeton foliosus, Sparganium americanum,* and *Vallisneria americana. Nymphaea odorata* (leaves and petioles) inhibited 78 % of lettuce seedling radicle growth and 98 % of *L. minor* frond production. *Brasenia schreberi* inhibited 82 % of lettuce seedling radicle growth and 68 % of *L. minor* frond production. These results suggest that *N. odorata* and *B. schreberi* are both highly inhibitory and are therefore candidates for use in aquatic weed management. Results also suggest that the simple lettuce seedling assay is a reasonable first "easy" one to use in an attempt to determine allelopathic potential of aquatic plants.

HISTORICAL REVIEW

Use of allelopathic terrestrial plants has received considerable attention in agriculture as a weed management strategy. More than 80 years ago, SCHREINER and REED (1907) reported the detrimental effect *Sorghum bicolor* L. has on succeeding crops. In their 1978 review, PUTNAM and DUKE proposed the exploitation of allelopathy for weed control and identified specific crop residues as potential sources of selective herbicides. Later work (1983) by PUTNAM and DEFRANK showed that total weed biomass and mass of several indicator species were consistently reduced by residues of barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.) and sorghum (*Sorghum*

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bicolor L.). Careful controls, including use of populus wood shavings as a control mulch to reduce light by 97 % and to reduce the temperature by about 1 °C while at the same time ensuring that no toxins were released, allowed the separation of physical and chemical aspects of the residue effects. Bioassays of aqueous plant extracts suggested that phytotoxins are directly released by the plant residues. Because of the economic importance of crop plants, many other workers have examined aspects of exploitation of allelopathy for terrestrial weed control. Much less work has been reported concerning allelopathic aquatic plants, but, as WALLER (1987) suggested in his recent ACS monograph, "... work on dwarf spikerush gives hope that we might manage aggressive aquatic plants with nonweedy allelopathic species". In fact, OBORN *et al.* (1954) suggested 34 years ago that replacement of an undesirable species by a desirable one could provide a long-term, site-specific method of aquatic weed management. Although OBORN *et al.* give no experimental details or references, they stated that "laboratory evidence over a two-year period indicated that either or both of these plants (dwarf arrowhead, *Sagittaria subulata*, or needle spikerush, *Eleocharis acicularis*) growing in association with the taller more obnoxious pond weed, *Potamogeton*, would, over a period of time, crowd out the pond weed growth." Current aquatic weed management programs include chemical (herbicides), biological (use of insects, animals, and allelopathy), and mechanical (plant harvesting) methods as well as integrated methods. The use of allelopathy as a method of aquatic weed management is not likely to replace other control methods, but certainly it should be complementary to them. The economic benefits are not likely to be of the magnitude of those encountered with terrestrial crop plants; nevertheless, keeping public waterways free of unwanted vegetation is an important ongoing process.

A thorough search of the literature revealed a dearth of reports concerning allelopathic aquatic plants. Only about 20 papers actually reported field observations or experimental evidence in support of their existence. Only a limited number of such plants have been identified, and of these, the allelopathic potential of dwarf spikerush, *Eleocharis coloradoensis*, has been examined more extensively than that of any other aquatic plant. In 1980 FRANK and DECHORETZ planted *Potamogeton nodosus* and *P. pectinatus* in *E. coloradoensis* sod and also in aquaria to which were daily added 500 ml of leachate from *E. coloradoensis* sod. Numbers of new shoots of *Potamogeton* were significantly reduced in each case, and there was also a reduction in biomass. *Potamogeton pectinatus* was more sensitive to the influence of *E. coloradoensis* than was *P. nodosus*. That same year, YEO (1980) reported his observations of *E. coloradoensis*, over a 12-year period, in several water systems in California. He found that *Potamogeton pectinatus*, *P. nodosus*, *P. pusillus*, and *Najas guadalupensis* were, within two years, displaced by *E. coloradoensis*. Two species of *Elodea (canadensis* and *nuttallii*) were displaced, but required longer than two years. NICHOLS and SHAW (1983) also reported that *E. coloradoensis* was able to displace other aquatic plants. In addition to these field observations, YEO and

THURSTON in 1984 conducted outdoor competitive experiments. Planting schemes included seven individual species of aquatic weeds, each co-planted with *E. coloradoensis*, and *E. coloradoensis* alone. Dry masses of all seven of the aquatic weeds were reduced when the plants were grown with *E. coloradoensis*. For six of the seven, dry mass was less than 35 % of the dry masses of aquatic weeds in monoculture. More recently, ASHTON *et al.* (1985) examined the allelopathic potential of organic compounds leached from axenically cultured *E. coloradoensis*. They separated the leached organics into several fractions and separately bioassayed them using the aquatic plants *Hydrilla verticillata* and *Potamogeton pectinatus*, as well as tomato cell cultures and lettuce seedling roots, as the bioassay target species. Some fractions were found to be inhibitory to all of these target species. Certainly, these several studies give good evidence that *E. coloradoensis* possesses allelopathic properties. Although *E. coloradoensis* has been studied most extensively, other aquatic plants also show evidence of allelopathic properties. It is also clear that many different assay systems have been used to examine allelopathic potential. If aquatic plants are to be used as part of a biocontrol program, then some method of determining which plants have the greater activity is necessary. Obtaining useful information about the nature of allelochemicals derived from aquatic plants requires a method to select which plants should be investigated first, that is, which are the most promising source of allelochemicals. Because determining the chemical nature of allelochemicals is an arduous task, the selection of which species to investigate is an important one. Bioassay techniques that are rapid, reliable, and biologically meaningful are needed.

MATERIALS AND METHODS

Plant Processing

Field-collected, fresh plants were washed free of debris and dried to the "drip-dry" stage. Voucher specimens were deposited in the University of Southern Mississippi herbarium. A 200-g aliquot of the drip-dry plants was thoroughly blended with 200 ml of distilled, deionized water, and the resulting pulpy mixture was refrigerated for 24 to 72 h. Refrigeration retarded bacterial growth; longer times enhanced the extraction of organic materials. The mixture was filtered through cheese cloth to remove the majority of the cellulosic material, through filter paper to remove smaller particulate matter, and finally through a 0.45 µm Millipore filter to render the solution sterile. In all cases except one, the entire plant was subjected to extraction. The exception was *Nymphaea odorata* which was divided into two portions: leaves and petioles; rhizomes and roots.

Bioassays

Details of the lettuce seedling and the *L. minor* bioassay procedures are published elsewhere (ELAKOVICH and WOOTEN 1988).

RESULTS AND DISCUSSION

In order to develop a list of aquatic plants which might have value in aquatic plant management, we selected 16 aquatic plants for study (Table 1). The plants were chosen for a variety of reasons. *Brasenia schreberi* and *E. acicularis* were selected because they had been reported to be allelopathic. *Eleocharis obtusa* was included because of the importance of *Eleocharis* in allelopathy. *Myriophyllum aquaticum* was selected because it is a desirable plant of the same genus as the nuisance plant, *M. spicatum* (Eurasian watermilfoil). Some plants were selected because of their observed potentially allelopathic activity as determined by their field-observed growth habits. *Hydrilla* and Eurasian watermilfoil were selected because they are the two major weeds in the southeastern United States, and we wished to compare their acitivity with that of more desirable plants. All plants were collected from field populations, aqueous extracts of each were prepared, and the sterilized extracts were subjected to bioassay.

TABLE 1
Plant species selected for this study

1. *Brasenia schreberi*
2. *Cabomba caroliniana*
3. *Ceratophyllum demersum*
4. *Eleocharis acicularis*
5. *Eleocharis obtusa*
6. *Hydrilla verticillata*
7. *Juncus repens*
8. *Limnobium spongia*
9. *Myriophyllum aquaticum*
10. *Myriophyllum spicatum*
11. *Najas guadalupensis*
12. *Nymphaea odorata*, leaves and petioles
13. *Nymphaea odorata*, rhizomes and roots
14. *Nymphoides cordata*
15. *Potamogeton foliosus*
16. *Sparganium americanum*
17. *Vallisneria americana*

Selection of the bioassay system is an important process. As mentioned earlier, the assay systems used in literature reports are not uniform. They range from the use of specific aquatic plants in competition studies to root growth inhibition of lettuce, radish, tomato, or cucumber by whole plant extracts or leachates of various plant parts. Tomato cell cultures have even been used as the target system in the testing of axenically cultured *Eleocharis coloradoensis*. We selected the lettuce seedling bioassay for initial screening because it is a widely used assay in allelopathy, which is experimentally simple, has short time requirements, and is very sensitive. Because we also wished to use an aquatic plant as the target plant (lettuce being a terrestrial plant), we also employed the *Lemma minor* assay developed by EINHELLIG *et al.* (1985).

We chose initially to examine aqueous extracts of our selected plants under the premise that in aquatic systems water-soluble allelochemicals are the more important ones. FISCHER *et al.* (1987) has very elegantly shown that natural micelles may aid in the transport of water-insoluble allelochemicals, which suggests that such compounds may also be important. In the lettuce seedling bioassay, the effect of the aqueous plant extracts was measured at three extract concentrations: one, five, and ten ml of extract per test plate. The results are shown graphically in Fig. 1. Extracts of six plants inhibited greater than 77 % of lettuce seedling growth. These are, in order of inhibition, *Nymphaea odorata* rhizomes and roots, *Juncus repens*, *Vallisneria americana*, *Brasenia schreberi*, *Ceratophyllum demersum*, *Eleocharis acicularis*, and *Nymphaea odorata* leaves and petioles. Of these, *N. odorata* rhizome and root extract was the most active, with 95% inhibition of lettuce radicle growth. *Ceratophyllum demersum* extracts brought about the greatest inhibition (66 %) at the lowest extract concentration. Interestingly, both *B. schreberi* and *V. americana* are strongly inhibitory at the highest concentration, but are stimulatory at the lowest concentration tested. Statistical analysis showed these findings to be statistically significant. RICE (1984) has suggested that many, perhaps most, plant growth inhibitors may be growth stimulators at some much lower concentrations, and extracts of these two plants appear to exemplify his suggestion.

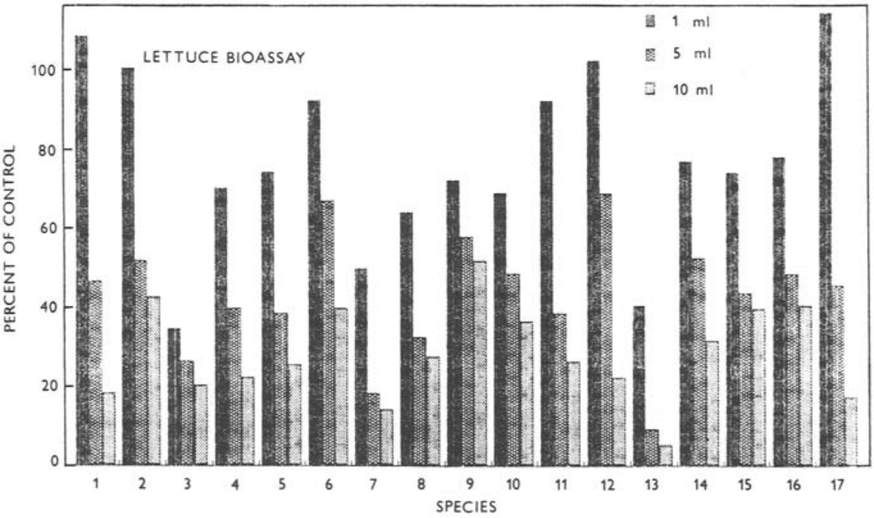

Fig. 1. Results of lettuce seedling bioassay. Plant species numbered as in Table 1. Concentrations indicated are volume of extract per test plate and correspond to concentrations of 25, 125, and 250 ppt, respectively.

The *Lemna minor* assay developed by EINHELLIG *et al.* (1985) provides an assay involving an aquatic plant as the target species. In this assay system, axenic *L. minor* is cultured in 1.5 ml of growth medium in 24-well sterile tissue culture plates. Each 24-well test plate allows six replications of each of three test concentrations and a control. The lettuce seedling assay easily allows 20 (or more) test plants for each concentration. Given the immense variability of plant growth, 20 replications is certainly far superior to six. The reproducibility of the *L. minor* assay in our hands was variable. We ran all assays in duplicate. Some duplicate assays agree well, some do not. It is likely that the small replication number contributes significantly to the lack of correspondence. The obvious advantages of this assay system over the lettuce seedling system are that an aquatic plant is employed, a whole plant system is used, and only small amounts of compounds are required. Disadvantages include a longer assay time with more complex set-up procedures and smaller replication numbers.

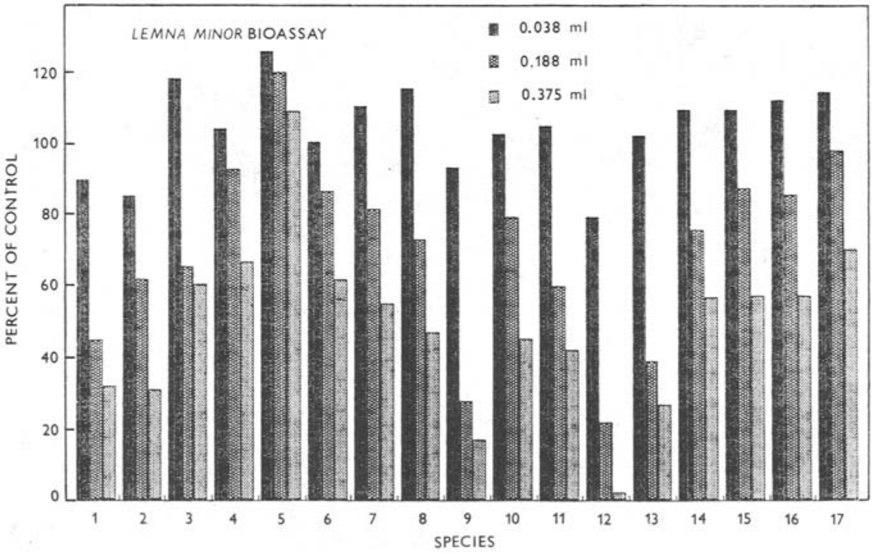

Fig. 2. Results of *L. minor* bioassay. Graph reflects the mean of the means of the two replicates. Plant species are numbered as in Table 1. Concentrations indicated are volume of extract per test well, and correspond to concentrations of 20, 100, and 200 ppt, respectively.

Results from the *L. minor* assay are given in Fig. 2. Extract concentrations were selected to compare to the concentrations used in the lettuce seedling assay (at 2×10^4, 10×10^4, and 20×10^4 ppm crude aqueous extract). Extracts of five plants inhibited 68 % or more of *L. minor* frond reproduction. These are (in order of inhibitory properties) *Nymphaea odorata* leaves and petioles, *Myriophyllum aquaticum*, *N. odorata* rhizomes and roots, *Cabomba caroliniana*, and *Brasenia schreberi*. Comparing these results with those from the lettuce seedling assay shows that *Nymphaea odorata* (rhizomes and roots) was the most inhibitory plant tested by lettuce seedling assay, inhibiting 95 % of seedling radicle growth at the highest extract concentration. *N. odorata* (rhizomes and roots) also inhibited *L. minor* frond reproduction by more than 70 %. *Nymphaea odorata* (leaves and petioles) was the most inhibitory plant tested by *L. minor* assay, inhibiting 98 % of frond production. This same plant extract inhibited lettuce seedling radicle growth by 78 %. *Brasenia* *schreberi* inhibited 82 % of lettuce seedling radicle growth and also inhibited 68 % of frond reproduction in one *L. minor* assay. Less activity was exhibited in the second (duplicate) *L. minor* assay. These results suggest that both *N. odorata* and *B. schreberi* are excellent candidates for aquatic plant management. On the other hand, *Juncus repens*, the second most active plant by lettuce seedling assay, does not appear highly allelopathic by *L. minor* assay.

It is not possible from this limited list of aquatic plants and these results to suggest the best plants for use in allelopathic management of aquatic weeds. Our list of 16 selected plants needs to be expanded to include other native species that have allelopathic potential. We are convinced by this work that although it would be ideal to assay plants against a specific species selected for eradication, such specific assays would first need to be developed, and such development would be time consuming, so the lettuce seedling assay is a useful one. The results of our two assay systems are not exact, but there is enough similarity to indicate that most plants which prove highly active by the lettuce seedling assay are also highly active by the *L. minor* assay. The much greater simplicity of the lettuce seedling assay makes it a good choice for initial screening assays such as these. It would, of course, be interesting to apply both of these assays to additional plant species to further test the seeming agreement of the two systems. Plants which are shown to be highly active in both assays should be good candidates for replacement species in aquatic plant management and also may be appropriate for further study of allelochemicals. Obviously there is more work to be done!

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